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REVIEW

Structural requirements of pyrroloquinoline quinone dependent enzymatic reactions

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Abstract

On the basis of crystal structures of the pyrroloquinoline quinone (PQQ) dependent enzymes methanol dehydrogenase (MDH) and soluble glucose dehydrogenase (s-GDH), different catalytic mechanisms have been proposed. However, several lines of biochemical and kinetic evidence are strikingly similar for both enzymes. To resolve this discrepancy, we have compared the structures of these enzymes in complex with their natural substrates in an attempt to bring them in line with a single reaction mechanism. In both proteins, PQQ is located in the center of the molecule near the axis of pseudo-symmetry. In spite of the absence of significant sequence homology, the overall binding of PQQ in the respective active sites is similar. Hydrogen bonding interactions are made with polar protein side chains in the plane of the cofactor, whereas hydrophobic stacking interactions are important below and above PQQ. One Arg side chain and one calcium ion are ligated to the *ortho*-quinone group of PQQ in an identical fashion in either active site, in agreement with their proposed catalytic function of polarizing the PQQ C5-O5 bond. The substrates are bound in a similar position above PQQ and within hydrogen bond distance of the putative general bases Asp297 (MDH) and His144 (s-GDH). On the basis of these similarities, we propose that MDH and s-GDH react with their substrates through an identical mechanism, comprising general base-catalyzed hydride transfer from the substrate to PQQ and subsequent tautomerization of the PQQ intermediate to reduced PQQ.

Keywords: catalytic mechanism; electron transfer; glucose dehydrogenase; methanol dehydrogenase; PQQ; quinoprotein; X-ray structure

Quinoproteins form a class of enzymes, which use quinone cofactors to oxidize a wide variety of alcohols and amines to their corresponding lactones/aldehydes (Duine, 1991; Davidson, 1993; Anthony, 1996). Proteins containing the noncovalently bound cofactor 2,7,9-tricarboxy-1H-pyrrolo[2,3-f]quinoline-4,5-dione (PQQ, Fig. 1) constitute the best known and largest quinoprotein subgroup (Duine, 1991). The X-ray structures of the PQQ-containing methanol dehydrogenase (MDH) and soluble glucose dehydrogenase (s-GDH) are known (Ghosh et al., 1995; Xia et al., 1996; Oubrie et al., 1999a, 1999b).

MDH is found in the periplasmic space of methylotrophic bacteria (Anthony, 1996). It oxidizes methanol to formaldehyde with concomitant reduction of PQQ to PQQH₂. The natural electron acceptor is the acidic cytochrome *c_L* (Anthony, 1993). MDH is an $\alpha_2\beta_2$ heterotetramer with a molecular weight of 140 kDa. Each

α -domain (571 residues in the *Methylophilus* W3A enzyme) contains one PQQ molecule and one calcium ion, which is essential for activity (Richardson & Anthony, 1992). Several other PQQ-dependent proteins, including membrane-attached glucose dehydrogenase (m-GDH), are homologous to the α -subunit of MDH (Cleaton-Jansen et al., 1990) and are expected to have a similar fold (Cozier & Anthony, 1995; Cozier et al., 1995; Jongejans et al., 1998).

In contrast, s-GDH from *Acinetobacter calcoaceticus*, of which homologs have recently been found in several other Gram-negative bacteria (Oubrie et al., 1999b), has a completely different amino acid sequence (Cleaton-Jansen et al., 1988). This enzyme is a homodimer (Dokter et al., 1986), which binds one PQQ molecule and three calcium ions per monomer (50 kDa, 454 residues) (Olsthorn & Duine, 1996; Oubrie et al., 1999b). One of the calcium ions is required for activation of the cofactor, the other two are needed for functional dimerization of the protein (Olsthorn & Duine, 1996; Olsthorn et al., 1997; Oubrie et al., 1999b). s-GDH oxidizes a wide range of mono- and disaccharides to the corresponding ketones and it is able to donate electrons to several artificial electron acceptors such as N-methylphenazonium methyl sulfate (Olsthorn & Duine, 1996) and electroconducting polymers (Ye et al.,

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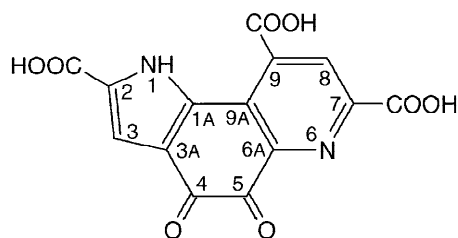


Fig. 1. Chemical structure of PQQ (2,7,9-tricarboxy-1H-pyrrolo[2,3-f]quinoline-4,5-dione).

1993). The physiological electron acceptor of s-GDH is unknown, although electrons can be transferred from PQQH₂ to cytochrome b₅₆₂ (Dokter et al., 1988).

For PQQ-dependent enzymes, two different reaction mechanisms are normally considered (Frank et al., 1989; Anthony et al., 1994; Anthony, 1996; Olsthoorn & Duine, 1998): The first mechanism is addition-elimination, comprising general base-catalyzed proton abstraction, the formation of a covalent substrate-cofactor complex, and subsequent product release (Fig. 2A). The second possible reaction mechanism involves general base-catalyzed proton abstraction in concert with direct hydride transfer from the substrate C1 atom to the PQQ C5 atom, and subsequent tautomerization of the cofactor to PQQH₂ (Fig. 2B). The rate-limiting step for s-GDH and MDH is the conversion of the enzyme-substrate complex into reduced enzyme and product (Frank et al., 1988; Olsthoorn & Duine, 1998). Moreover, the deuterium isotope effect is about 6 for both enzymes (Duine & Frank, 1980; Frank et al., 1988; Olsthoorn & Duine, 1998), and the spectral properties of the enzymes are similar, resembling those of PQQ adducts (Frank et al., 1988; Frank et al., 1989; Olsthoorn & Duine, 1998). Therefore, it may be anticipated that s-GDH and MDH catalyze their respective redox reactions in an identical manner. In addition, NAD⁺-dependent and flavin-dependent dehydrogenases catalyze the conversion of alcohols into carbonyls in an identical fashion, even though their substrates and substrate binding clefts may be completely different. However, the MDH-PQQH-methanol complex supports the addition-elimination mechanism (Xia et al., 1999), whereas the direct hydride transfer mechanism was put forward after analysis of the s-GDH-PQQH₂-glucose complex (Oubrie et al., 1999c). This discrepancy prompted us to compare the enzyme-substrate complexes of both enzymes. Our results show that PQQ, calcium, the putative general bases and the oxidizable hydroxyl groups of the substrates are in a comparable arrangement in both active sites, suggesting that the enzymes catalyze redox reactions in an identical fashion.

Three-dimensional structures of MDH and s-GDH

MDH is an $\alpha_2\beta_2$ heterotetramer. Each α -domain has a β -propeller fold, composed of eight four-stranded antiparallel β -sheets. The eight β -sheets are arranged in a circular fashion and give rise to an eight-fold axis of pseudo-symmetry (Fig. 3A). An amino acid segment called the Trp-docking motif recurs eight times in the sequence and forms a girdle of stabilizing interactions in the three-dimensional (3D) structure (Ghosh et al., 1995; Xia et al., 1996). The β -subunit (74 residues in MDH from *M. W3A*) has an extended structure without hydrophobic core. The function of this

latter domain is not well understood (Ghosh et al., 1995; Xia et al., 1996).

s-GDH from *A. calcoaceticus* is a homodimer. Each domain has a β -propeller fold similar to the α -subunit of MDH, but it is constructed from six rather than eight four-stranded antiparallel β -sheets, resulting in a considerably smaller structure (Fig. 3B). An internal sequence repeat with a stabilizing function is present in s-GDH, but the conserved amino acids give rise to a different type of stabilizing interaction compared to that in MDH (Oubrie et al., 1999b). Thus, the 3D structures of MDH and s-GDH are different although they belong to the same family of structurally related proteins. Since also the amino acid sequences of these two proteins are completely different, any similarities in their respective active sites are expected to have a functional role.

Cofactor conformation in the enzyme-substrate complexes of MDH and s-GDH

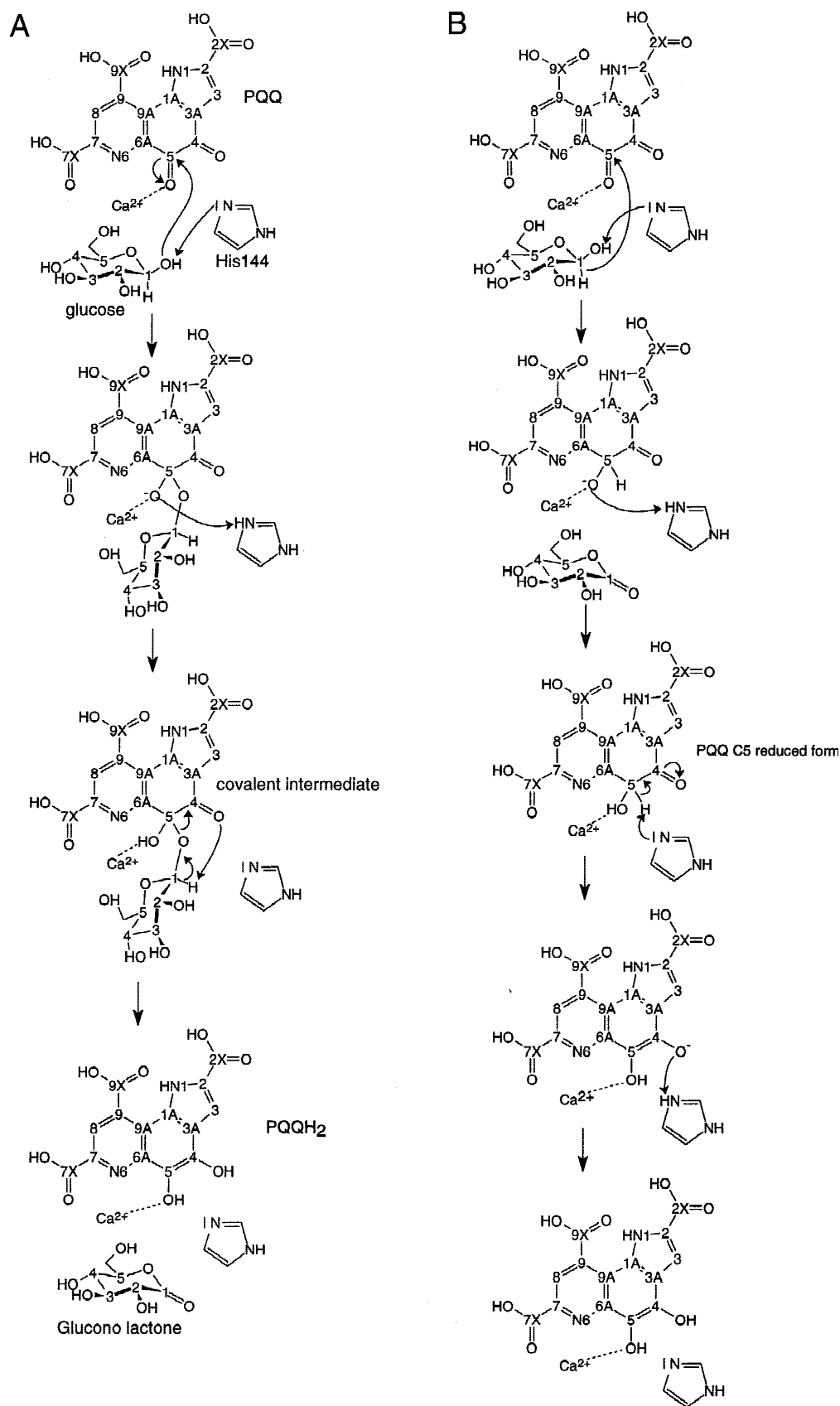
In all MDH structures, PQQ is present as the free radical semi-quinone (PQQH) (Xia et al., 1999). The PQQH C5 atom has a tetrahedral conformation and is out of the molecular plane. As a result, the central ring of PQQH in MDH is distorted from planarity. In contrast, the corresponding ring of PQQ in the 1.5 Å resolution structure of s-GDH is planar because all atoms have a sp² hybridization (Oubrie et al., 1999a).

In the s-GDH-PQQH₂-glucose complex, PQQH₂ represents the reduced form of PQQ (Oubrie et al., 1999c). Comparison of s-GDH-PQQH₂ with a high-resolution structure of s-GDH-PQQ (not shown) shows that the oxidized and reduced cofactor forms bind in an almost identical fashion to the enzyme. Only small differences with respect to planarity exist: PQQ deviates slightly from planarity, whereas PQQH₂ is completely planar (Oubrie et al., 1999a, 1999c).

PQQ binding sites of MDH and s-GDH

In MDH, PQQH is buried in a chamber close to the central channel of the β -propeller near the N-terminal end of the A-strands. It is sandwiched between the indole ring of Trp327 and the vicinal disulfide bridge formed by Cys103 and Cys104 (Fig. 4A) (Ghosh et al., 1995; Xia et al., 1996). In the plane of the cofactor, many hydrogen-bonding interactions are made between PQQH and polar amino acid side chains (Fig. 4C). Furthermore, PQQH ligates the active site calcium ion through its N6, C5, and O7A/B atoms. The remaining calcium ligands are provided by the side chains of Asn255 and Glu171.

In s-GDH, PQQ resides in a large cleft near the sixfold pseudo-symmetry axis (Oubrie et al., 1999a, 1999c). The cofactor is bound upside-down compared to the binding of the cofactor in MDH (Fig. 4). It stacks on a largely flat and hydrophobic surface, made up from the side chains of Gln231, Gln246, Ala350, and Leu376 (Fig. 4B). The upper side of PQQ is exposed to the solvent. All equatorial interactions between the cofactor and the s-GDH side chains are through hydrogen bonding and ion-pair interactions (Fig. 4D). The binding of calcium to the N6, O5, and O7B atoms of PQQ is identical to that in MDH. The other calcium ligands are different (side chains of Asn255 and Glu171 in MDH, main-chain oxygen atoms of Gly247 and Pro248, and two water molecules in s-GDH). The presence of an Arg residue close to the *ortho*-quinone group is conserved, although the precise orientation with respect to the quinone group is slightly different in both enzymes.



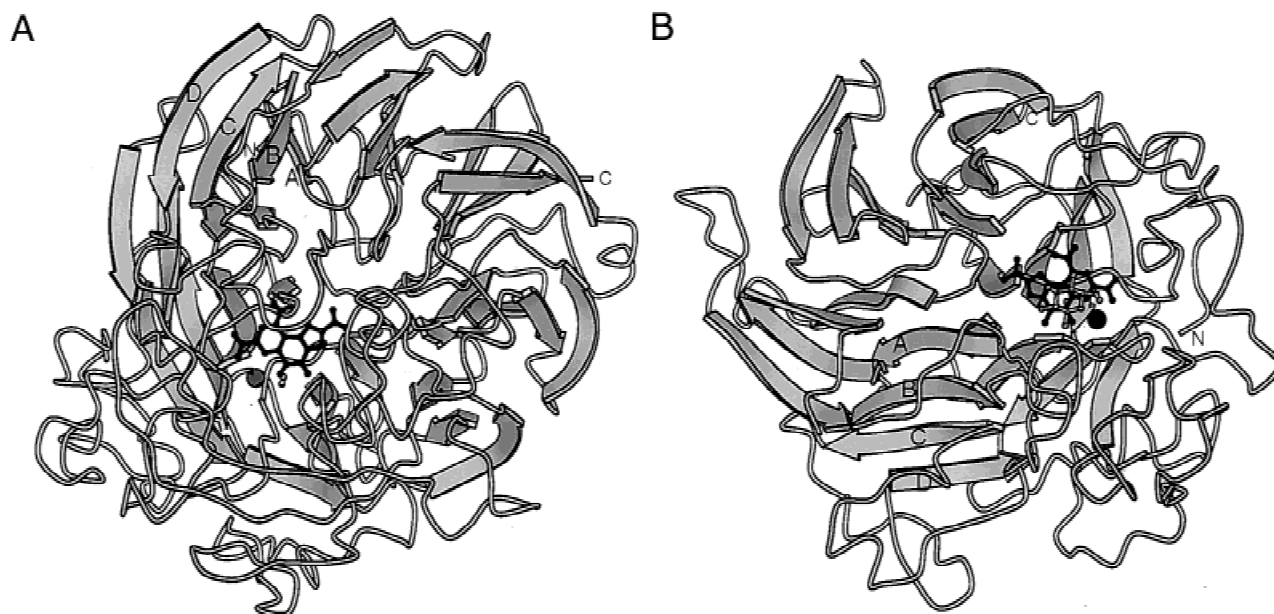


Fig. 3. Ribbon diagrams of (A) MDH and (B) s-GDH monomers. Due to the absence of structural homology (this figure) and the mirrored mode of binding of PQQ in the active sites (Fig. 4), the 3D structures of MDH and s-GDH were superimposed on the basis of the central ring of PQQ with the program O (Jones et al., 1991). MDH and s-GDH both have a β -propeller fold, composed of eight and six four-stranded antiparallel β -sheets, respectively. In each β -sheet, the β -strands are labeled from the inside to the outside of the protein, as is indicated in one β -sheet of each structure. The N- and C-termini are indicated. (A) Calcium, PQQH and methanol, and (B) calcium, PQQH₂, and glucose are shown in ball-and-stick representation. This and following figures were produced with MOLSCRIPT (Kraulis, 1991).

Thus, the nature of the interactions between the cofactor and the protein side chains in MDH and s-GDH is dictated by the features of PQQ. These interactions are hydrophobic above and below the cofactor plane, whereas the equatorial interactions are polar.

No functional disulfide bridge is present in the active site of s-GDH

In MDH, the vicinal disulfide bridge formed by Cys103 and Cys104 makes a hydrophobic contact with PQQ. The peptide group between the two vicinal cysteines has an unusual nonplanar *trans* configuration. The disulfide bridge has been implicated in the stabilization or protection of PQQH, which is an intermediate in the stepwise process of the transfer of two electrons from PQQH₂ to two cytochrome *c_L* molecules (Avezoux et al., 1995).

In contrast, there is no equivalent to this vicinal disulfide bridge in s-GDH. An alternative disulfide bridge, formed between the two single cysteine residues Cys338 and Cys344, is too far from PQQ to be involved in stabilization of the possible PQQH intermediate. Moreover, since the two cysteines are not present in sequences that are homologous to s-GDH (Oubrie et al., 1999b), they do not seem to have an important function.

Substrate binding

In the ternary MDH-PQQH-methanol complex, methanol is bound above the PQQH cofactor. Both carbon and oxygen atoms of methanol have high *B*-factors, indicating that the substrate is bound with a low occupancy and a high flexibility. The methyl group of methanol is buried in a small hydrophobic cavity in the protein

interior that is formed by the side chains of Trp259, Trp531, Leu547, and the vicinal disulfide bridge. The hydroxyl group is hydrogen bonded to the side chain of Asp297 at a distance of 3.1 Å. The distances of the hydroxyl group and the methyl group to the PQQH C5 atom are 3.2 and 3.9 Å, respectively (Fig. 5A) (Xia et al., 1999).

On the other hand, the glucose binding site in s-GDH is in a wide and solvent accessible crevice, which is located directly above PQQH₂ (Oubrie et al., 1999c). The carbon and oxygen atoms of glucose have *B*-factors that are only slightly higher than those of PQQ and the protein ligands. This suggests that the substrate may be bound with a high occupancy and low flexibility. In agreement with the absolute β -anomer preference of s-GDH, only β -glucose is bound in the crystals, even though the natural mixture of α - and β -anomers was used in the soaking solutions. Glucose docks onto the PQQH₂ surface, making extensive hydrophobic interactions (Fig. 5B). Other, less extensive hydrophobic interactions are made between Leu169 and the glucose C1 atom, and Tyr343 and Trp346 form a hydrophobic patch within van der Waals distance to the glucose O4 hydroxyl and C5 hydroxymethyl groups. Hydrogen bonds are made between the glucose O2 atom and the side chains of Gln76 and Asp143, and also between the glucose O1 atom and the side chains of His144, Gln168, and Arg228. The O3 hydroxyl group of glucose does not make any interaction with the protein (Oubrie et al., 1999c), which explains the lack of specificity of s-GDH toward this position (Olsthoorn & Duine, 1998). Interestingly, the distance between the glucose C1 atom and the PQQ C5 atom is only 3.2 Å (Oubrie et al., 1999c). Thus, glucose and methanol have a similar geometry above the cofactor, with their C1 atoms within 4 Å of the PQQ C5 atom and their oxidizable

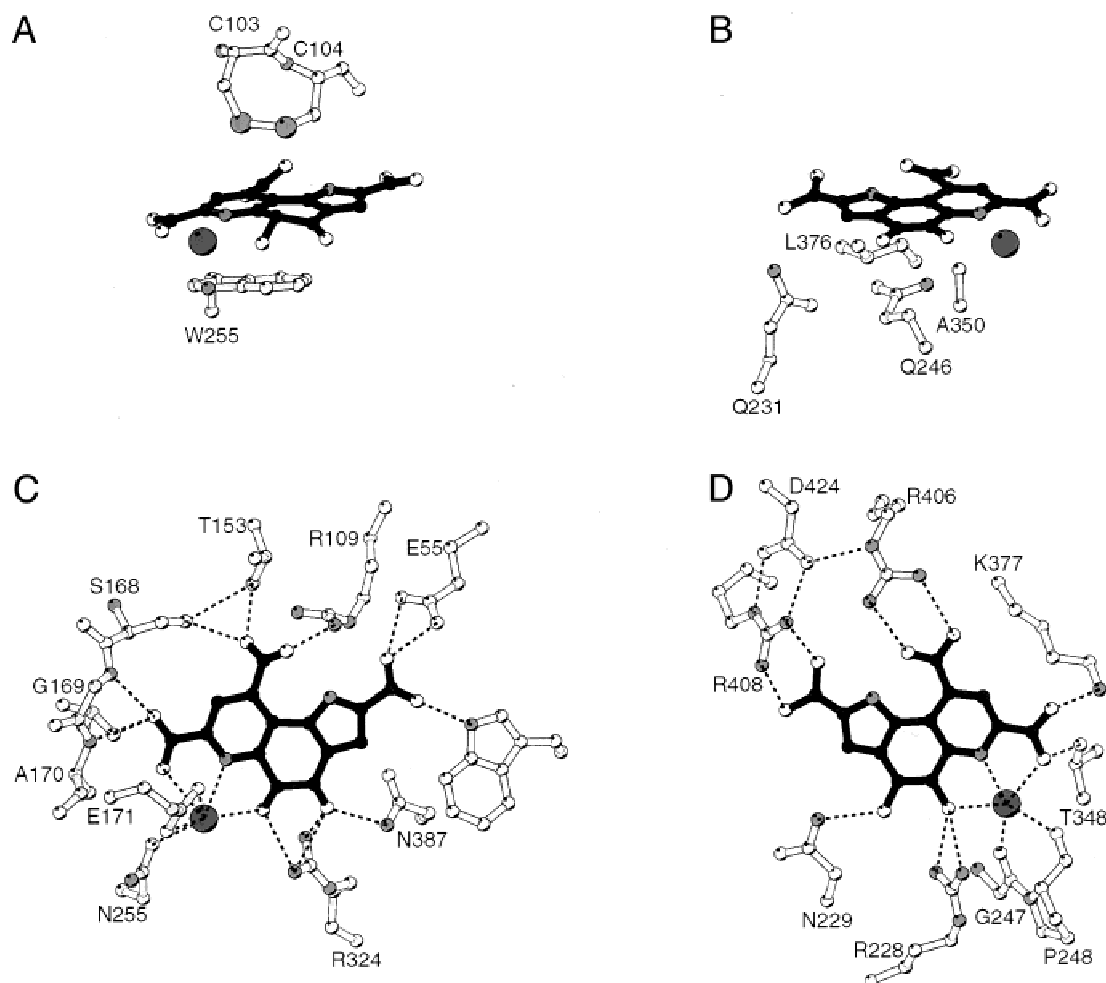


Fig. 4. A, B: The hydrophobic interactions of PQQ in the active sites of (A) MDH and (B) s-GDH, respectively. **C, D:** Hydrogen bonding interactions between the cofactor, calcium, and protein side chains in the active sites of (C) MDH and (D) s-GDH, 70° rotated with respect to A and B, respectively. The orientation of PQQH in C corresponds to that of MDH in Figure 3A, and the orientation of PQQH₂ in D corresponds to that of s-GDH in Figure 3B. Hydrogen bonds are indicated by dotted lines. The calcium ion is shown as a large grey sphere. Oxygen, carbon, and nitrogen atoms are shown in increasing darker shades, starting from white. Carbon atoms of the cofactor are depicted in black. Amino acid side chains are labeled.

hydroxyl groups within hydrogen bonding distance to a basic amino acid side chain (Asp297 in MDH and His144 in s-GDH).

The catalytic role of calcium

MDH and s-GDH are inactive in the absence of calcium (Richardson & Anthony, 1992; Olsthoorn & Duine, 1996; Olsthoorn et al., 1997). Addition of calcium converts the PQQ-containing apo-enzymes into the active *holo*-enzymes (Goodwin et al., 1996; Olsthoorn & Duine, 1996; Olsthoorn et al., 1997). Calcium can be replaced by divalent cations such as strontium and barium in both enzymes, resulting in active enzyme preparations with altered kinetic properties (Adachi et al., 1990; Harris & Davidson, 1994; Goodwin & Anthony, 1996; Goodwin et al., 1996; Olsthoorn et al., 1997). These experiments clearly indicate that calcium is required for catalysis in both MDH and s-GDH. In agreement with a catalytic role, the active site calcium is bound in an identical fashion

to the N6, O7A, and O5 atoms of PQQ in both enzymes (Fig. 4). However, neither methanol nor glucose is directly ligated to the metal ion. Therefore, calcium does not seem to be involved in the binding of the substrate, in contrast to what has been proposed previously (Anthony et al., 1994; Zheng & Bruice, 1997). Instead, calcium may polarize the PQQ C5-O5 bond together with the active site Arg residue, resulting in a partial negative charge on the O5 atom and a partial positive charge on the C5 atom (Zheng & Bruice, 1997; Olsthoorn & Duine, 1998; Oubrie et al., 1999c). Consequently, the C5 atom is expected to be highly reactive toward nucleophiles (Ghosh et al., 1995; Zheng & Bruice, 1997; Olsthoorn & Duine, 1998; Oubrie et al., 1999c; Xia et al., 1999). Recently, a binding study with methylhydrazine in s-GDH crystals revealed that this inhibitor forms a covalent complex with PQQ at its C5 position. This study confirmed that the C5 atom is indeed the most electrophilic atom of PQQ in the active site of s-GDH and indicated that calcium most likely contributes to catalysis by the polarization of the C5-O5 bond of PQQ (Oubrie et al., 1999a).

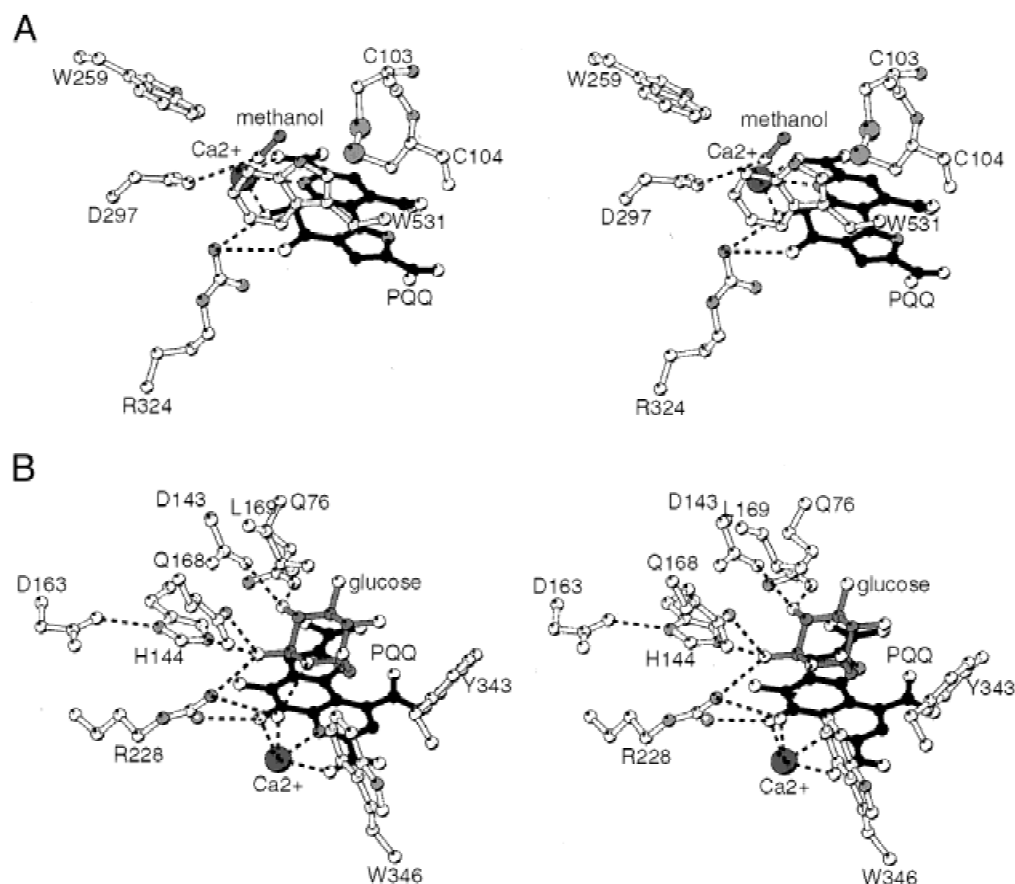


Fig. 5. Stereoview of substrate binding in the active sites of (A) MDH and (B) s-GDH. Hydrogen bonds are indicated by dotted lines. The same color coding is used as in the previous figure.

The catalytic bases

In the ternary MDH-methanol complex, Asp297 is within hydrogen bonding distance of the hydroxyl group of methanol (Fig. 5A) (Xia et al., 1999). Similarly, the imidazole group of His144 in s-GDH forms a hydrogen bond with the oxidizable O1 hydroxyl group of glucose (Fig. 5B) (Oubrie et al., 1999c). These basic amino acid side chains have been suggested to act as general bases that initialize the reaction by abstracting a proton from the substrate's hydroxyl group (Ghosh et al., 1995; Oubrie et al., 1999c; Xia et al., 1999). The participation of a general base in the reaction has first been postulated by Frank et al. (1989) on the basis of inhibition experiments of MDH with cyclopropanol.

The imidazole group of His144 is hydrogen bonded to Asp163 (Fig. 5B). This Asp-His dyad system in s-GDH is reminiscent of the Asp-His-Ser catalytic triad, present in serine proteases (recently reviewed by Dodson & Wlodawer, 1998). In the latter triad, the His functions as the general base by accepting the seryl O γ proton. The Asp keeps the imidazole group in the right orientation and maintains the Ne2 atom in an unprotonated state (Sprang et al., 1987; Kumar et al., 1994; Dodson & Wlodawer, 1998). A similar function could be envisaged for the residues in the putative catalytic dyad in s-GDH. The Asp163-His144 hydrogen bond could stabilize the conformation of the imidazole group. It may also keep the imidazole Ne2 atom in an unprotonated state, enabling His144 to act as the general base. Hence, although definite conclusions

await a mutational analysis of the active site bases, it seems likely that Asp297 in MDH and His144 in s-GDH participate in catalysis by abstracting a proton from the respective substrates.

The catalytic mechanism

PQQ-dependent enzymes oxidize their substrates either through an addition-elimination or a direct hydride transfer mechanism (Fig. 2) (Anthony, 1996; Olsthoorn & Duine, 1998). Below, the likeliness of these catalytic mechanisms is discussed in relation to the available biochemical, kinetic, and structural data.

The addition-elimination mechanism

The addition-elimination mechanism has been put forward on the basis of four arguments: (1) In solution, covalent adducts of free PQQ can readily be formed with various compounds such as water and methanol (Dekker et al., 1982; Itoh et al., 1993). (2) Absorption and fluorescence spectra of certain covalent adducts of isolated PQQ are almost identical to spectra of the transient MDH-methanol and s-GDH-glucose complexes (Frank et al., 1988; Olsthoorn & Duine, 1998). (3) Studies with isolated PQQ showed that methanol, ethanol, and 2-propanol can be oxidized to the corresponding aldehyde *via* this mechanism (Itoh et al., 1997; Itoh & Fukuzumi, 1998). (4) In the ternary MDH-PQQH-methanol complex, the

methanol hydroxyl group is closer to the PQQ C5 atom than the methyl group (Xia et al., 1999). In view of the recent progress in obtaining structural data on quinoprotein-substrate complexes, we can now discuss the validity of each of the four arguments in a structural context herein after.

1. There is no direct structural evidence that covalent adducts of substrates such as methanol, glucose, or compounds such as water are actually formed in the active sites of enzymes. In the crystal structure of *holo*-s-GDH, for instance, the absence of residual electron density close to the PQQ C5 atom indicates that PQQ is not hydrated in this enzyme. The essentially non-fluorescing character of oxidized s-GDH supports this observation. Moreover, also the other characterized PQQ-dependent proteins, including MDH, are hardly fluorescent when they are in the oxidized state, indicating that none of them is significantly hydrated (J. A. Duine, pers. comm.). The reactivity of the PQQ C5 atom toward nucleophiles is thus lower in enzymes than in solution.
2. The validity of the second argument was questioned by Olsthoorn and Duine (1998) who argued that the C5 reduced form of PQQ, which would be the intermediate after direct hydride transfer, may also give rise to similar spectra as the transient enzyme-substrate complexes. Thus, spectral properties cannot discriminate between the two proposed mechanisms.
3. The central line of evidence that an addition-elimination reaction takes place was inferred from spectroscopic measurements on a nonenzymatic model system with PQQ in solution (Itoh et al., 1997; Itoh & Fukuzumi, 1998). The effects of various cations and PQQ analogs on the oxidation of methanol, ethanol, and 2-propanol were taken as proof that the pyrrole ring of PQQ facilitates general base-catalysis by the O4 carbonyl oxygen atom and that this substantiated the addition-elimination mechanism (Itoh et al., 1997; Itoh & Fukuzumi, 1998). However, this reaction was carried out in anhydrous MeCN and not in an aqueous solution. This may result in dramatically different conditions in terms of basicity and accessibility of the reactive groups. Therefore, even though the reaction in solution may proceed through an addition-elimination mechanism, this does not necessarily mean that the same mechanism could operate in MDH and/or s-GDH.
4. In the ternary MDH-PQQH-methanol complex, the C5 atom of PQQH has a tetrahedral conformation. It is displaced out of the molecular plane toward the substrate-binding site (Xia et al., 1999). The displacement of the C5 atom distorts the planarity of the central ring of PQQH, which is much less planar than the corresponding ring of PQQ in the high-resolution structure of s-GDH. Simultaneously, this displacement may have forced methanol to move upward as well. As a result, methanol may be bound in a nonfunctional and therefore possibly unstable binding mode. In agreement with this proposition are the high *B*-factors of its carbon and oxygen atoms in the 3D structure (Xia et al., 1999). Thus, the ternary MDH-PQQH-methanol complex may not be a reliable mimic of a transient MDH-PQQ-methanol complex.

In addition to the concerns of the validity of the four arguments for the addition-elimination mechanism, the ternary enzyme-substrate complex of s-GDH is not compatible at all with such a

mechanism. The O1 hydroxyl group is at a distance of 3.34 Å from the PQQ C5 atom, but it points away from that atom (Fig. 5) (Oubrie et al., 1999c). Therefore, the glucose O1 atom, negatively charged after proton abstraction by His144, would have to be reoriented before a covalent bond can be formed with the PQQ C5 atom. This can only be achieved through a significant rearrangement of the glucose in the active site, necessitating the breaking of several hydrogen bonds and/or salt bridges between protein side chains and the glucose O1 oxide and O2 hydroxyl groups. The activation energy for such a rearrangement seems considerable, and a reaction along this path is thus expected to be slow, which is incongruous with the high turnover number of s-GDH. Alternatively, a rearrangement of the glucose anion in the active site could be achieved by the opening of the cyclic ring. This would allow the α -enantiomer of glucose to be formed. The O1 oxide atom of this enantiomer would then be expected to point down toward the PQQ C5 atom, in which orientation it could perform a nucleophilic attack on the C5 atom of PQQ. However, Olsthoorn and Duine (1998) have demonstrated that the enzyme is absolutely specific for the β -enantiomer of glucose, not for the α -enantiomer. Moreover, the cyclic form of glucose fits snugly in the active site pocket, and it is unclear how s-GDH could catalyze the ring opening of the substrate.

The direct hydride transfer mechanism

The constellation of the reactive groups in the ternary s-GDH-PQQH₂-glucose complex seems ideal for direct hydride transfer from the glucose C1 atom to the PQQ C5 atom. The former atom is positioned directly above and within 3.2 Å of the latter atom (Oubrie et al., 1999c). Since the C1 hydrogen atom is in an axial position in β -glucose, it should point down in the direction of the PQQ C5 atom. Therefore, an axially positioned hydride ion would have to be transferred over a distance of only 1.2 Å for covalent addition to the C5 atom. This would explain the absolute preference of s-GDH for the β -enantiomer (Olsthoorn & Duine, 1998). The optimal orientation of the two reactants might allow for a fast reaction to occur. Kinetic experiments indeed indicate that the oxidation of glucose commences with a fast step, which results in the formation of a fluorescing intermediate (Olsthoorn & Duine, 1998). Olsthoorn and Duine (1998) suggested that this intermediate could be the C5 reduced form of PQQ. For s-GDH, all biochemical and kinetic data are in agreement with the general base-catalyzed direct hydride transfer mechanism.

For MDH, the biochemical and kinetic data such as the deuterium isotope effect and the fluorescing character of the intermediate are consistent with a hydride transfer mechanism, as was noticed previously (Anthony, 1996). It remains to be clarified that the MDH-PQQH-methanol complex is also compatible with such a mechanism. As was discussed above, the planarity of the central ring of PQQH is distorted, and this may have forced methanol to bind in a nonfunctional manner. PQQ is expected to have a more planar conformation, with the C5-O5 bond approximately in the molecular plane. This may cause methanol to bind in a fashion parallel to PQQ. Only small changes are necessary for such rearrangements, for which enough space seems available. In fact, a movement of methanol of about 1 Å would result in a structure with the methyl group above the PQQ C5 atom and a strong hydrogen bond between the hydroxyl group and the putative general base Asp297. Such an arrangement would be comparable to that of PQQH₂-glucose and would favor hydride transfer.

Hence, after a detailed analysis of the enzyme-substrate complexes of MDH and s-GDH and a reconsideration of the available biochemical and kinetic data for these two enzymes, it is highly questionable that PQQ-assisted enzymatic catalysis proceeds through addition-elimination. Instead, we propose that s-GDH, MDH, and possibly other PQQ-dependent proteins react with their substrates via a mechanism that includes general base-catalyzed proton abstraction, direct hydride transfer from the substrate to PQQ, and tautomerization of C5-reduced PQQ to PQQH₂.

Conclusions

PQQ-dependent MDH and s-GDH have distinct amino acid sequences and 3D structures. Both structures have a β -propeller fold, consisting of eight (MDH) and six (s-GDH) β -sheets, respectively. In both protein structures, PQQ is located at the same side of the β -propeller fold. In either active site, PQQ makes hydrogen-bonding interactions with polar protein side chains in the plane of the cofactor, whereas hydrophobic stacking interactions are important below and/or above PQQ. The positive charges of the active site calcium ion and one Arg side chain accumulate close to the PQQ C5-O5 bond, suggesting this bond to be polarized. This would result in a partial positive charge on the PQQ C5 atom, making this atom highly reactive toward nucleophiles. In the ternary enzyme-substrate complexes, methanol and glucose are bound in a similar position, with the substrate C1 atom above the PQQ C5 atom, and the substrate O1 atom within hydrogen bond distance of the putative general bases Asp297 (MDH) and His144 (s-GDH). This similar constellation of the reactants in the active sites, together with the similarity of the kinetic details of both enzymes, suggests that MDH and s-GDH catalyze their respective redox reactions through an identical mechanism. On the basis of a detailed analysis of the enzyme-substrate complexes of MDH and s-GDH with respect to the available biochemical and kinetic information, we propose that PQQ-assisted enzymatic catalysis proceeds through a mechanism comprising general base-catalyzed proton abstraction in concert with direct hydride transfer.

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